

## THE TETANUS BACILLUS AS AN INTESTINAL SAPRO-PHYTE IN MAN.\*

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It is well recognized that *Bacillus tetani* is widely distributed in nature, but the rôle of man as a carrier of this organism has received little attention. In the literature it is repeatedly stated that man may carry the tetanus bacillus in his digestive tract, the statement being apparently based on the work of Pizzini and also on the fact that this offers the best explanation for some idiopathic cases of tetanus and for the cases following typhoid fever, dysentery, and hemorrhoid operations.

Pizzini (1) injected suspensions of feces from 100 individuals into guinea pigs and in five instances obtained symptoms of tetanus. Ten of the individuals were hostlers, and the feces of three of them produced tetanus symptoms, while of the remaining 90, who were peasants, the feces of only two produced these symptoms. The natural conclusion drawn from this work was that man seldom carried the tetanus bacillus and that those who are in intimate contact with animals that are known to be carriers of this organism were more apt to be infected. During his extensive work on tetanus Tulloch (2) became interested in the digestive tract as a source of infection in abdominal tetanus. He studied the feces of twenty-one men returned from overseas service and in seven, or 33 per cent, isolated tetanus bacilli. From the feces of thirty-one civilians in England he obtained this organism in five instances, or 16 per cent. Tulloch's results are surprising not because they differ from Pizzini's, for his methods were undoubtedly better, but because they show such a large percentage of infections of the human intestinal tract. It is difficult to explain why in the numerous studies that have been made on the anaerobic flora of the human digestive tract we find no mention of the isolation of tetanus bacilli, but this may be due to interest in other organisms rather than to the fact that these bacilli were absent.

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Before Tulloch's paper came to our attention we became interested in the presence of tetanus bacilli in the digestive tract through a case on the service of Dr. A. S. Taylor in which the best explanation of the source of infection seemed to be a fecal contamination of a bed-sore. In the present paper we wish to report the results of the examination of the feces of 78 individuals and to bring out some evidence which makes us feel that tetanus bacilli are the normal inhabitants of the intestinal tracts of a certain number of individuals.

We should like to emphasise the fact that the examinations were, with one exception, made on the feces of male Chinese, and that they, as did Tulloch's overseas men, live in more intimate contact with the soil than does the average man in Europe or America, so that our findings may not be duplicated in the Occident. While the individuals examined were hospital patients, we tried to secure specimens from recently admitted cases on both the medical and surgical wards and the results would probably be the same if normal individuals were examined.

#### *Methods.*

The stools were collected in clean but not sterilized bedpans and a portion was transferred to sterile Petri dishes which were sent to the laboratory. Approximately a 10 per cent suspension was made in sterile salt solution, the cotton plug was pushed down, and a rubber stopper inserted into the mouth of the tube. The tube was then immersed in a water bath at 80°C. and kept there for 20 minutes to destroy the non-spore-bearing organisms, after which 1 cc. of the suspension was transferred to sugar-free bouillon containing a piece of sterile rabbit kidney or spleen in a fermentation tube as recommended by Smith (3) for the cultivation of anaerobes. After 4 days incubation films were made from the sediment in the tube and searched for forms characteristic of tetanus bacilli. If the terminal round spore-bearing forms were not found the stool was called negative, while if they were found the stool was not called positive until the bacilli had been isolated and it was shown that a spasm-producing toxin was formed that was neutralized by tetanus antitoxin.

The organisms were isolated by heating the impure cultures to 80°C. for 20 minutes transferring to sterile bouillon plus tissue in a fermenta-

tion tube, and plating when the tetanus-like forms predominated. This procedure was repeated until what appeared to be a pure culture was obtained, when it was plated once more and transplants were made from well isolated colonies. This apparently pure culture was grown in sugar-free bouillon plus tissue, in a fermentation tube, for 10 days, the bulb fluid was discarded, and the branch fluid was centrifugalized until it was clear. Field or white mice were then inoculated subcutaneously with this clear fluid, two receiving 0.001 cc., two 0.01 cc., two 0.01 cc. plus approximately one unit of tetanus antitoxin, and two 0.1 cc. plus the same amount of antitoxin. All of the cultures that we have called *Bacillus tetani* produced characteristic spasms and death in the mice injected with 0.001 cc. and in the mice injected with 0.1 cc. plus approximately one unit of tetanus antitoxin there were no spasms and the mice lived at least 5 days, when the experiment was discontinued.

#### *Discussion of Methods.*

This method involved a great deal of work as it was often very difficult to get the organisms in pure culture though they were plated repeatedly. The injection of mixed culture was tried and it was found that at times spasms and death did not occur when characteristic tetanus-like organisms were present although when these organisms were isolated they proved to be tetanus bacilli. The results of the injections of impure cultures made on 2 different days are given in Table I.

It will be seen that some of the mice died so early that a diagnosis could not be made, though spasms might have been obtained in these animals had smaller amounts been injected. Other mice showed spasms and a definite diagnosis could be made, but the animals inoculated with seven out of the sixteen cultures lived 5 days or more and showed no symptoms of tetanus, though it was afterwards proved that the culture with which they were inoculated contained tetanus bacilli. It is evident, therefore, that there are organisms which interfere with the production of, or destroy tetanus toxin and that the injection of mixed cultures is not a reliable method to use in the detection of tetanus bacilli.

TABLE I.

*Effect of the Injection, into White Mice, of the Centrifugalized Branch Fluid from Fermentation Tubes Containing Sugar-Free Bouillon Plus Tissue Inoculated with Heated Suspensions of Feces and Incubated 10 Days.*

The sediment from all of these tubes showed organisms having the morphology of tetanus bacilli and from all of them *B. tetani* was later isolated in pure culture.

Patient No.	Mouse.	Effect of the injection of 0.5 cc. of culture.	Result.
340	A	Dead next morning. Cause?	?
	B	" " " " ?	
411	A	" in 20 hrs. Generalized tetanus.	+
	B	Died immediately after inoculation.	
346	A	Dead in 20 hrs. Generalized tetanus.	+
	B	" " 20 " " "	
399	A	" " 48 " " "	+
	B	" " 48 " " "	
226	A	Alive at the end of 2 wks. No spasms.	—
	B	" " " " " 2 " " "	
231	A	Dead in 48 hrs. Generalized tetanus.	+
	B	" " 48 " " "	
317	A	Alive at the end of 2 wks. No spasms.	—
	B	" " " " " 2 " " "	
266	A	Dead in 20 hrs. Generalized tetanus.	+
	B	" " 20 " " "	
227	A	" next morning. Cause?	+
	B	" in 24 hrs. Generalized tetanus.	
172	A	Alive at the end of 2 wks. No spasms.	—
	B	" " " " " 2 " " "	
1426	A	Dead in 24 hrs. Generalized tetanus.	+
1496	A	Alive at the end of 2 wks. No spasms.	—
1456	A	" " " " " 2 " " "	—
1495	A	Dead in 48 hrs. Generalized tetanus.	+
1484	A	" " 5 days. No spasms. Death was probably due to an organism other than tetanus.	—
1494	A	Alive at the end of 2 wks. No spasms.	—

Bengtson and McCoy (4) have shown that the direct inoculation of animals with smallpox vaccine containing tetanus bacilli is not nearly as delicate a test for this organism as the injection of 10 day fermentation tube cultures, and our results show that this cultural test cannot be relied upon, at least when testing for tetanus bacilli when other intestinal organisms are present.

As all the earlier work on the presence of tetanus bacilli in the intestinal tracts of various animals and in soil is based either on results obtained from direct inoculation or the injection of impure cultures, it must be assumed that if the work were repeated and tetanus-like organisms isolated, the percentage of positive findings from street dirt, cattle, horse feces, etc., would probably be greater than those already reported.

We have made only one culture from each individual and only 0.1 gm. of stool was cultured, and we have relied upon the microscopic examination of the sediments from the fermentation tube cultures for the first separation of positive from negative stools. We therefore feel that our percentage of positive cases may be too low, but in a paper to be published shortly we shall show that from the blood evidence can be obtained of an intestinal infection, and that the blood findings and stool examinations, as we have made them, agree very closely.

With regard to the microscopic examinations it is worthy of note that in over thirty stools in which we found tetanus-like organisms on the first examination in only one instance did we fail to isolate organisms that produced toxin. We obtain the sediment from the fermentation tube for microscopic examination by introducing a capillary pipette down to the bend of the tube, thus obtaining more of the anaerobes than it is possible to get with a platinum loop. Practically all of the film examinations were made after the tubes had been inoculated 4 days, but lately it has been found that tetanus bacilli are more numerous in tubes that have been incubated 5 or 6 days. In examining films we rely mainly on the morphology of the organisms and pay little attention to the Gram staining, as on the 4th or 5th day most of the tetanus bacilli are negative to Gram's stain.

The results of the microscopic examinations have been so consistent that it is strongly urged that films should be made from anaerobic

cultures of vaccine virus on the 5th or 6th day of incubation. If organisms having the morphology of tetanus bacilli are found in these films a negative result following the injection of the mixed culture into animals should not be taken to indicate that tetanus bacilli are absent but the tetanus-like organisms should be isolated and tested for toxin production before the virus is used on man.

*Results of the Examination of Stools from 78 Individuals.*

Using the above methods we examined during April and May, 1921, forty-three stools of patients in the hospital and from eleven, or 25.6 per cent, obtained tetanus bacilli. In August, September, and October of the same year we examined thirty-five more stools and from sixteen, or 45.7 per cent, isolated tetanus bacilli. In all we have therefore examined 78 stools and from 34.7 per cent have obtained organisms which in their morphology and toxin-producing properties were tetanus bacilli.

An examination of the histories of the twenty-seven positive cases reveals little that bears on the problem. With the exception of one American they were all male Chinese of the poorer class. The youngest was 5 and the oldest 44 years, and their average age was 20.3 years. Twelve of them had been in the hospital less than 10 days and eight more than 30 days. They had a variety of diseases, and from our point of view they may be taken to represent the average Chinese of the lower class.

While our results might indicate that carriers of tetanus bacilli are more numerous at certain seasons of the year we feel inclined to believe that the higher percentage of positive cases in the last group is due to the fact that we were more expert in the examination. We should therefore prefer to group our cases together and to say that in Peking among the lower classes at least one out of every three individuals is discharging tetanus bacilli in his feces.

Our results agree with Tulloch's findings in overseas men, and we predict that similar results will be obtained in other regions where great numbers of people live in close contact with the soil. In Peking this contact is very intimate, for during the winter and spring it is very dry and windy so that dust from the streets as well as from the fields outside the city (fertilized with human feces) is constantly being taken into the gastrointestinal tract.

*Tetanus Bacilli as Intestinal Saprophytes.*

We naturally became interested in the much disputed question as to whether the tetanus bacillus is primarily an inhabitant of the digestive tract as claimed by Sormani, or whether Vincent's contention is correct that its main habitat is outside the body and that spores found in the feces are those taken in with the food that have passed through the tract without germinating.

Sormani (5) based his contention on the facts that he found tetanus bacilli in the feces of a variety of animals, and in a dog infected by feeding, the feces showed tetanus spores 16 days later though it was fed on sterile food and was prevented by a muzzle from taking other food or licking contaminated surfaces. He reasoned that conditions for growth were more favorable in the body than outside and concluded that the former was its natural habitat. Vincent (6) admitted that tetanus spores might be found in the digestive tract but he failed to find them. He also showed that there was a destruction of the spores when they were introduced into the stomachs of rabbits or guinea pigs whose pylori had previously been ligated, and that the intestinal secretions prevented the development of tetanus bacilli in the test-tube. He also failed to find evidence of multiplication of tetanus bacilli in the intestinal tract of rabbits 12 hours after feeding tetanus spores. Since he was unable to show that tetanus bacilli multiplied in the digestive tract he concluded that their natural habitat must be outside the body. Bombicci (7) and von Esmarch (8), however, found that tetanus bacilli disappeared from putrefying mixtures, which is against Vincent's view that they are saprophytic organisms. Noble (9) examined the feces of a horse, a natural carrier, over a period of 7 months during the first 4 months of which time tetanus spores were found on every examination, while two negative horses in the same stable remained negative during this period though they were receiving the same food.

We had rather exceptional facilities for studying the question as to whether tetanus bacilli multiplied in the digestive tract as the patients in the hospital were receiving practically sterile food. The reducing valves for the steam-cookers had not been installed so that the steamed bread and rice that were the principal articles of their diet were cooked under a pressure of from 30 to 50 pounds of steam. The vegetables were carefully washed, peeled, and then boiled for at least 20 minutes. While a few tetanus spores might survive the latter process this is hardly likely, and we felt that if we could show that patients who had been in the hospital for some length of time eliminated spores, this would indicate that the organisms were multiplying in their intestinal tracts.

While the hospital food is practically sterile the patients' friends undoubtedly break the rules of the institution and bring in food from the streets which might contain tetanus spores. This is a factor that could not be controlled but the chances of infection are much less than in the recently admitted patients.

Eleven stools were received from patients that had been in the hospital for some length of time. The results of the examination are given in Table II where it will be seen that from seven of these eleven cases, or 63.6 per cent, that had been in the hospital 23 days or longer tetanus

TABLE II.

*Presence of Tetanus Spores in the Stools of Patients Who Had Been Receiving Practically Sterile Food for a Considerable Time.*

August 9, 1921.

Patient No.	Length of time in hospital.	Tetanus spores in feces.
	<i>days</i>	
231	36	Positive.
299	27	Negative.
317	23	Positive.
266	30	"
227	39	"
172	45	"
152	47	"
144	28	Negative.
226	41	Positive.
238	40	Negative.
138	59	"

Patient 172 was an American.

bacilli were isolated from the feces. In addition, the bacillus was found in a case that has been in the hospital 105 and in another that has been in the hospital 86 days. Our series of eleven cases is so small that we do not care to emphasize the fact that the percentage of carriers (63.6 per cent), is nearly twice that found in our complete series (34.7 per cent), but these results seem to indicate that the tetanus bacilli are multiplying in the digestive tracts of these patients.

We next tried to estimate the number of tetanus spores in the stools of three carriers by making known dilutions of the stools and determining the highest dilution in which tetanus bacilli could be found.

The stools were received in sterile bedpans, weighed, mixed, and from accurately weighed portions 10 per cent suspensions were made in sterile salt solution. These suspensions were shaken vigorously to break up clumps and a portion was transferred to other sterile test-tubes that were corked and then heated to kill the non-spore-bearing organisms. A series of known dilutions was then made and from each 1 cc. was transferred to each of two fermentation tubes containing sugar-free bouillon plus tissue. After 4 days incubation films were made and examined for tetanus-like organisms. From the tube containing the highest dilution of feces that showed tetanus-like organisms anaerobic plates were made and in each case pure cultures were obtained that produced toxin which caused the characteristic

TABLE III.

*Estimation of the Approximate Number of Tetanus Spores Eliminated by Three Individuals.*

Patient No.	Age.	Diagnosis.	Length of time in hospital.	Weight of stool.	No. of tetanus spores,	
					Per gm. of feces.	Eliminated.
	yrs.		days	gm.		
158	5	Kala-azar.	105	74	10,000	740,000
374	9	Tubercular osteomyelitis.	35	258	10,000	2,580,000
932	13	Tuberculosis.	27	200	1,000,000+	200,000,000

symptoms of tetanus in mice and which was neutralized by tetanus antitoxin.

The results obtained from the examination of these three cases are given in Table III. It so happened that children were chosen for the test so that their stools were small, but it is inconceivable that this great number of spores was taken into the intestinal tract and passed through without germinating. The tetanus bacilli must have been established and growing in the intestinal tract.

#### DISCUSSION.

A discussion of the methods used in detecting tetanus bacilli in mixed cultures is to be found in the body of the paper and will not be repeated here. It is sufficient to say that the isolation of the bacillus and the demonstration that the culture forms a spasm-producing toxin

that is neutralized by tetanus antitoxin is the only reliable method. The injection of mixed cultures gives uncertain results, for while tetanus bacilli may be present, other organisms may interfere, with toxin production.

The results show that one-third of the male population in the vicinity of Peking carries tetanus bacilli in the digestive tract and that the bacilli probably are multiplying there. Man thus plays a large rôle in the distribution of the bacillus, for it is not uncommon to see human feces deposited on the streets, and human feces are used to fertilize the fields, so that dust from the streets and fields must contain great numbers of spores that come from man. One wonders why all of the population are not carriers of the bacillus, and it may be that at some time in their life they are but that other organisms crowd the tetanus bacillus out or make conditions unfavorable for its growth.

It is difficult to say just how these large numbers of tetanus carriers influence the case incidence of tetanus. Foreign doctors see very few cases of infection, probably not more than in European countries. In our own hospital with a large out-patient clinic and nearly 2,000 in-patients per annum there were six cases of tetanus in 1919 and three cases in 1920, and physicians who have practised in China for years have told us that they rarely see cases of tetanus. We have been unable to obtain a record of tetanus following typhoid fever or dysentery, though these diseases are extremely common. Rose (10), however, reports several cases of tetanus following typhoid in Germany, and there are numerous reports in the literature of cases following hemorrhoid operations, so that it is evident that the bacilli in the digestive tract are at times able to enter the body and produce their toxin. We have seen one case of tetanus following a fall that ruptured the intestine but did not produce an external injury. It is evident, however, that foreign physicians in China see in adults very little tetanus due directly or indirectly to an intestinal infection. This may be owing to the fact that the Chinese recognize the disease as a fatal one and that patients are afraid to come to foreign hospitals as they want to die at home. On the other hand, it may be that carrying the tetanus bacillus in the digestive tract for some time produces a relative immunity to this organism. In a paper to be published shortly we shall produce evidence which supports this latter view.

While we have no accurate statistics we think that tetanus neonatorum is extremely common. Lennox (11) in questioning hospital patients as to their past history found that of 8,468 children born, 61 per cent died of convulsions or madness. It is quite probable that many of these deaths were due to tetanus, the infection coming from the filthy dressings used on the cord, and while the figures are only approximate they give some indication of the great loss of life which is in part, at least, associated with the spreading of tetanus bacilli by man.

#### CONCLUSIONS.

1. The only reliable method that can be used for the detection of tetanus bacilli is the culturing of the suspected material, the isolation of tetanus-like organisms and the demonstration that the pure cultures form a spasm-producing toxin that is neutralized by tetanus antitoxin.

2. Using this method we have demonstrated tetanus bacilli in 34.7 per cent of stools from 78 individuals in Peking.

3. The tetanus bacillus is growing in the digestive tract, for it is present in individuals who have been on a practically sterile diet for a month or more, and one individual may eliminate several million spores of tetanus bacilli in a single stool.

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